TECHNICAL STATUS REPORT
Contract No. NASw-870

Period 4 February 1969 to
4 May 1969

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1.0 BACKGROUND

This document reports progress on tasks defined under Contract No. NASw-870, Modification No. 6. Under this agreement, Space/Defense Corporation (herein after "the contractor") has continued to develop and evaluate the performance of single and multi-specimen respirometers. The ultimate goal of this program is to provide instruments suitable for performing research concerned with biorhythms and the mechanisms responsible for the establishment and control of observed rhythmic activity.

The configuration of these instruments is specifically oriented toward the conduct of biological rhythmicity studies in the space environment. The potato tuber has been chosen as the experimental subject on the basis of the relative simplicity in the basic requirements for the maintenance of a viable organism over extended periods, and secondly, because of a great mass of baseline data already collected from this organism in the earthbound environment.

The program thus far has been responsible for the successful development of single-specimen respirometric systems selected for flight in the Apollo Applications Program. Currently, development work focuses on multi-specimen systems more suitable for other potential space experiment applications. The progress of this work is described under paragraph 2.0, below.

In addition to these biorhythmic studies, a concurrent, second phase effort has been initiated to establish the feasibility of developing an experimental population of fishes which can be abnormally oriented relative to earth gravity, with the ultimate goal of relating the role of gravity to the regulation of biological systems. The progress of this program is reported in paragraph 3.0, below.

2.0 PROGRAM STATUS: PHASE I

2.1 General

During this reporting period, all respirometric development programs described in the contract are fully active. Further baseline data has been collected from the single specimen units and final assembly of the two multi-specimen has been completed. Continued problems in regulator hysteresis have led to a concerted effort to solve this persistent problem.

2.2 Technical

2.2.1 Single Specimen Respirometer

The single specimen respirometers have operated continuously for more than 13 months without breakdown. A new specimen collection scheme has resulted in maintaining an active organism in the respiration units for four months or longer. Mechanical malfunctions have not been observed except for those periods when the scrubbing of CO_2 was appreciably

impaired due to depletion of the scrubbing entity. At this time hysteresis in the second stage regulator becomes a major factor which perturbs the data appreciably. Performance of the new solenoid valve has been excellent with no valve malfunction occurring during the reporting period attributable to the design. One malfunction due to human error (test current overload) is reported. Three of the four units have been placed back on line to provide data base for comparison of "soft seat" regulators versus "hard seat" regulators. The units continue to operate satisfactorily.

2.2.2 Collaboration with Other Experimental Activities

During the reporting period active collaboration with the Ames Research Center BIOSATELLITE program group was maintained. A request for proposal for a potato respiration experiment based on modular design principles was received from ARC. A proposal utilizing NASw-870 technology will be supplied.

A Flight Opportunity Announcement was received from Hdq NASA and our P69-129 "Potato Respiratory Biorhythms" was submitted on 25 April 1969, proposing a potato respiration aboard a lunar orbiting subsatellite.

In addition, a request for proposal was received from the NASA AIBS group at the University of California, Berkely. Our proposal P69-130 "Potato Respirometric Rhythms: the Role of Geophysical Factors" was submitted on 30 April 1969.

2.2.3 Multi Specimen Unit Effort

This effort has not been actively pursued this reporting period because of the demands of the "hard seat" regulator.

2.2.4 Regulator Development Effort

The last quarterly report described the difficulties encountered using the "soft-seat" regulator and our plans for a "hard seat" development. During the reporting period, the proposed design was fabricated, assembled and tested. Initially, little success was enjoyed. Due to lack of concentricity in the ball guide channel, the ball failed to re-seat accurately after each regulator activation. Re-grinding the seat face and drilling out the ball guide channel only partially improved performance. Re-design using three new test configurations (stainless steel, quartz and synthetic sapphire) was pursued. It is believed that the close tolerances that can be maintained with these materials, combined with an improved ball guide configuration will improve performance of the "hard seat" regulator.

2.3 Plans for the Coming Period

Fabrication, assembly and test of the "hard seat" regulator using SS, quartz and sapphire seats will be accomplished. Performance at low flows will be compared to the soft seat devices now on line.

3.0 PROGRAM STATUS: PHASE II

3.1 General

To study the role gravity plays in regulating certain biological systems, teleost fishes were chosen because their orientation, relative to gravity, could be altered for prolonged periods of time. These lower vertebrates orient to gravity through integrating stimuli of external (visual) and internal (vestibular) origin. By surgical elimination of internal stimuli and altering the external cue, we hypothesized that this animal could be made to swim consistently in different orientations relative to the earth's gravitational force field, thereby providing a useful tool in studying the role gravity plays in neurohypophyseal hormone regulation.

Once fish are made to swim at predetermined axes relative to gravity we will determine the effects these altered orientations have on the hypothalamo-hypophyseal system in regards to the transport of neurohypophyseal hormones. The accurate assay of endogenous arginine-vasotocin (teleost vasopressin) in tissue sections taken from the hypothalamus, infundibulum and neurophyophysis of control and experimental fish must therefore, also be undertaken. To do this the following events must occur: arginine-vasotocin must be synthesized; antibodies to the exogenous hormone developed and fluorescently tagged; cross-reaction between endogenous hormone and fluorescent antibodies shown; and quantitative measurements made of changes in elaboration and/or transport of endogenous hormone in both control and experimental fishes.

As of the start of the reporting period we had successfully synthesized arginine vasotocin and have had success in inducing fish to persistently swim at altered angles relative to gravity. During the reporting period we continued work in both biochemical and surgical areas and the progress is described below.

3.2 Technical

3.2.1 Fish Orientation Studies

During this reporting period we have continued work with the angel fish (Pterophyllum scalare). Surgical procedures are now perfected and labarinthectomies can be consistently performed. After surgery, fish continuously orientate to a light cue rather than gravitational stimuli for as long as two months and exhibit normal behavior, eat well and interact with other fish.

3.2.2 Antibody Production

During the reporting period several approaches have been employed to induce antibody production to arginine vasotocin in our rabbits. To date, however, antibodies have not been produced in sufficient quantities for fluorescent work. We are hopeful that with continuous innoculations a sufficient antibody titer will develop and fluorescent tagging and assaying can then be achieved. It should be pointed out, however, that successful antibody production to small molecules can require several weeks. In the meantime, we are going to go ahead with

preliminary studies and employ standard histologic stains (Gomori's chrome hematoxylin) to assess the status of the hypothalamo-neurohypophyseal system of angel fish in altered gravitational fields.

3.3 Plans for the Coming Period

(a) Continue antibody production efforts.

(b) Antibody purification and conjugation when titers reach adequate levels.

(c) Initiate preliminary studies in which fish will be chronically exposed to altered gravitational orientations, sacrificed and their hypothalamo-hypophyseal systems sectioned and stained with Gomori's chrome hematoxylin to assess neurosecretory activity.

3.4 Related Activities

It should be noted that the techniques developed under this portion of NASw-870 are being employed in research for the U.S. Bureau of Commercial Fisheries, in an attempt to determine the role of the pituitary in the ale-wife die-off in Lake Michigan.